HER2 status in CTCs and DTCs in early stage breast cancer by fluorescence in situ hybridization (FISH) using a microfluidic cell enrichment and extraction platform (OncoCEETM).

**Background:**
Evaluation of HER2 gene amplification in CTCs and DTCs in early stage breast cancer by fluorescence in situ hybridization (FISH) using a microfluidic cell enrichment and extraction platform (OncoCEETM).

**Methods:** Blood (10ml) and BM (1-2ml) from patients with Stage T1 and T2 breast cancer was collected in OncoCEETM collection tubes. Mononuclear cells were recovered using a Percoll density gradient method, incubated with a mixture of 10 primary capture antibodies (Abs), and introduced into streptavidin coated OncoCEETM microchannels for tumor cell capture. For blood samples adjusted to a concentration of 1ug / ml for 30 minutes at room temperature. After centrifugation, secondary antibody was added to the cell pellet, incubated for 30 minutes at room temperature and centrifuged three times at 400 G for 10 minutes following washings with PBS/ Casein/ Arginine/ EDTA. The resulting cell pellet was then introduced to the microchannels (CEM microchip).

**Conclusion:**
1. The cell enrichment and extraction microfluidic platform (OncoCEETM) provides a sensitive approach for evaluation of HER2 in CTCs and DTCs. CTCs and DTCs lost HER2 gene amplified status in 52% and 83% of HER2 positive early stage primary breast cancer.
2. The clinical significance of alterations in HER2 status among CTCs and DTCs in early stage breast cancer needs further investigation.

**Results:**
Blood and BM from 68 patients (68 Blood, 54 BM, 54 matched blood and BM) with stage T1N1 (3), T1N0 (6), T1N0 (11), T2N1 (2), T2N2 (1), T2N2 (2) with HER2 + (n=7) and HER2- (n=61) breast cancers were studied. The 7 patients with HER2 + primary tumor had HER2+ DTCs in 3/7 (43%) and HER2 + CTCs in 1/8 (12%) patients. HER2 + DTCs and CTCs was detected in 12/47 (25%) and in 4/17 (23%) patients with HER2 + tumors. The discordance of HER2 status was observed in 14/84 CTCs and in 22% in DTCs.

1. Peripheral blood and BM from 68 patients staged as T1N0 in 41, T1N1 in 6, T2N1 in 11, T2N2 in 2, T2N3 in 2 patients with HER2 negative early stage primary breast cancer. 3. CTCs and DTCs lost HER2 gene amplified status in 52% and 83% of HER2 positive early stage primary breast cancer.
2. The clinical significance of alterations in HER2 status among CTCs and DTCs in early stage breast cancer needs further investigation.

**Methods**
- Peripheral blood and BM from 68 patients staged as T1N0 in 41, T1N1 in 6, T2N1 in 11, T2N2 in 2, T2N3 in 2 patients with HER2 negative early stage primary breast cancer.
- The primary invasive mammary tumor was positive for HER2 gene amplification in 7 patients and negative in 61 patients.
- HER2 gene amplified CTCs were detected by FISH performed on the intact CTCs in blood and BM from 68 patients with HER2 negative early stage primary breast cancer.
- HER2 gene amplified DTCs were detected in 3/7 (43%) HER2+ primary tumors and in 4/17 (23%) HER2 + primary tumors in BM.
- HER2 gene amplified DTCs were detected in 3/7 (43%) HER2+ primary tumors and in 4 of 17 (23%) HER2 primary tumors in BM.
- Her2 amplified status was evaluated by FISH as HER2 amplified status.
- Discrepancy of HER2 status as evaluated by FISH of intact CTCs and DTCs in the same tumors and patients provided useful information.
- Discrent HER2 status was contributed more often by DTCs in comparison to CTCs in patients with early stage breast cancer.

**Results**
- The 7 patients with HER2 + primary tumor had HER2+ DTCs in 3/7 (43%) and HER2 + CTCs in 1/8 (12%) patients. HER2 + DTCs and CTCs was detected in 12/47 (25%) and in 4/17 (23%) patients with HER2 + primary tumors. The discordance of HER2 status was observed in 14/84 CTCs and in 22% in DTCs.

**Conclusions**
1. The occurrence of circulating tumor cells (CTCs) in blood and disseminated tumor cells (DTCs) in bone marrow (BM) of patients with early and advanced breast cancer is well recognized. These tumor cells most likely play an important role in the complicated process of metastasis. Enumeration and characterization of these cells may increase early detection, improve design of personalized therapies, aid in the monitoring of treatment efficacy, enhance prognostic accuracy and advance our understanding of the biology of metastatic disease. Detection of CTCs and DTCs can be challenging because of their extreme rarity in the blood and BM. The microchannel techniques utilized for the detection of CTCs and DTCs do not allow detailed phenotypic and genotypic characterization of these cells. HER2/neu is the most commonly evaluated marker in breast cancer for determining eligibility of patients for treatment with humanized monoclonal antibody trastuzumab. Evaluation of HER2 gene amplification in CTCs and DTCs provides a sensitive approach for evaluation of HER2 status in patients with HER2 negative primary tumor and for consideration of this therapy in patients with HER2 positive primary tumor with otherwise HER2 positive CTCs and/or DTCs. In this study we report the discordance in HER2 status in CTCs and DTCs in early stage breast cancer as detected by fluorescence in situ hybridization (FISH) using a microfluidic cell enrichment and extraction platform (OncoCEETM).

**Introduction**

**Results**

**Methods**

**Abstract**

**Table 1**

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<tr>
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<th>DTCs</th>
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**Table 2**

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**Fig 1:** Illustration of the microfluidic platform (Biocept, Inc. San Diego) used in the study.

**Fig 2:** Illustration of a CK + and CD45- CTC. Note the numerous orange signals indicating increased copy numbers of HER2.

**Fig 3:** Illustration of a disseminated tumor cell (DTC) in bone marrow showing increased copy numbers of HER2 as evidenced by increased orange signals. The primary tumor was negative for HER2 gene amplification.